An Alcoholic Eosin and Methylene-Blue Staining Method.

By

R. K. S. Lim.

(From the Department of Physiology. Edinburgh University.)

Aqueous or alcoholic solutions of eosin and aqueous solutions of methylene-blue have long been individually and successively employed for the double staining of sections. (It is not intended here to deal with the mixed or combined staining method of Romanowsky (8), Ehrlich (1), and others.) The various methods now in use (Schafer (9), Sims Woodhead (10), Miller (5), etc.) may be generally described as follows:

Sections are stained with a water-soluble eosin solution for periods of 5–20 minutes or longer. They are then washed with water and brought into contact with a methylene-blue solution for a usually shorter time. After being again washed, they are differentiated and dehydrated in absolute alcohol, and subsequently cleared and mounted. Mallory and Wright (4) employ Unna's methylene-blue, which contains 1 per cent. of potassium carbonate. Richard Muir (6) uses a saturated solution of alcoholic eosin in rectified spirit, but drives off the alcohol by heat during the process of staining, leaving the eosin in watery solution. He then rinses in water and places in saturated potash alum for 3 minutes, subsequently decolorising with alcohol containing a trace of ammonia, and, after washing with water, stains with methylene-blue.

For blood-films the general method given above for sections

has been advantageously used after adequate fixation, e. g. with methyl-alcohol or with formol-alcohol (Gulland (2)), with the exception that the film is dried and mounted after the methylene-blue has been washed off. Türk stains with 0·5 per cent. eosin in 60–70 per cent. alcohol; he both dries and heats the film before applying methylene-blue solution.

Films of pus or of other exudates are also stained in a somewhat similar way (Muir and Ritchie (7)).

The results obtained by the above methods mainly depend upon the experience of the histologist. A successful preparation demonstrates well the nucleus, cytoplasm, and cell-granules, the latter especially if Richard Muir's method is used. The failure to obtain constant results is due to the difficulty of obtaining good differentiation. This difficulty is largely overcome with formalin-fixed tissues by the use of the following solutions, viz. 1 per cent. solution of alcohol-soluble eosin in rectified spirit, and 1 per cent. solution of methylene-blue in distilled water. In employing these for sections the latter are treated as follows:

1. Remove paraffin with xylol or benzol, then wash with absolute alcohol.
2. Pour on alcoholic eosin solution and leave for one minute.
3. Wash with water (distilled or tap).
4. Pour on methylene-blue solution and leave for one minute.
5. Wash with water; the sections should appear purplish.
6. Wipe slide dry with a fine cloth, leaving only the section moist.
7. Pour on absolute alcohol liberally to differentiate the staining, and immediately carry out the next step.
8. Pour on xylol or benzol to stop the differentiation and to clear the section for mounting.

During the manipulations the slide should be held obliquely in order that the reagents may run off.

The section may now be examined, and if found to be insufficiently differentiated, steps 7 and 8 may be rapidly

repeated; as a rule this is not necessary. When correctly differentiated and cleared, mount in dammar.

It is sometimes more convenient to have the methylene-blue and xylol in vessels large enough to accommodate a slide. In this case the slide should be agitated within the xylol until the section is cleared, when it may be mounted in dammar.

For blood or exudate films the same technique, if carried out implicitly, will give good results. The film is allowed to dry as slowly as possible in a cool place—the slower the better. When quite dry, staining may be commenced. Fixation is accomplished by the alcohol of the eosin solution, although for rapid work the film may be inundated with absolute alcohol for from 1 to 3 minutes prior to staining.

The above-described method has been used successfully upon sections for some time in this laboratory. It is rapid, simple, and certain, and is well suited as a routine procedure for most tissues. The preparations do not readily fade; some made in 1916 are as yet quite unchanged. It is particularly useful for the central nervous system and for the peripheral ganglia. It stains axis cylinders a deep red, Nissl's granules blue. Connective tissue is also stained an intense blue. It is valuable for glands, especially the pituitary, pancreas, and suprarenal. The oxyphil and basiphil granules of the anterior lobe of the pituitary are clearly differentiated; while in the pars nervosa (in man), free, coarse, greenish (basiphil?) granular masses, seemingly not identical with those described by Herring (3), are shown.

The results with blood-films are equal to, but are not claimed to be better than, a good Leishman (8) stained film. Nuclei of white blood-corpuscles are stained blue, granules according to their affinities, while the red blood-corpuscles come out bright red. In films of pus, etc., the bacteria are also stained blue.

In connection with this part of the subject, I wish to thank Dr. A. K. Towers for providing me with clinical material.

Literature.

1. Ehrlich and Lazarus.—'Die Anämie,' 1898, Wien.
2. Gulland.—'The Blood: a Guide to its Examination and to the Diagnosis and Treatment of its Diseases,' 1914, p. 30.
3. Herring.—'Quart. Journ. Exp. Physiol.,' 1908, vol. i, p. 149.
4. Mallory and Wright.—'Pathological Technique,' 1915, pp. 327–328.
5. Miller.—'Practical Pathology,' 1914, pp. 341–342.
6. Muir, Richard.—'Journ. Path.,' 1905–06, vol. xi, p. 373.
7. Muir and Ritchie.—'Manual of Bacteriology,' 1913, pp. 103–104.
8. Romanowsky.—'St. Petersburg med. Wochenschr.,' 1891. See also 'Enzykl. d. mikr. Tech.,' 1910, Bd. i, s. 121–122.
9. Schafer.—'Essentials of Histology,' 1916, p. 547.
10. Sims Woodhead.—'Practical Pathology,' 1912, p. 97.
11. Türk.—'Enzykl. d. mikr. Tech.,' 1910, Bd. i, s. 121.